Aust. Vet. J. 63: 282-285, 1986

Bioavailability

It is emphasised that this study considered only the permanent abnormality brought about by prolonged exposure of ewes to oestrogen. The type of abnormality in the cervix caused by a short-term exposure (1 to 40 days) of ewes to phyto-oestrogens (Adams 1977b) or to oestradiol-17β (Bell *et al* 1941) is quite different in nature from that described here. The short-term change resembles the usual effects seen in most other female mammals after exposure to oestrogen.

Clover-affected ewes in the present study were more severely affected that those exposed to oestradiol-17β (Table 1). This was expected, because the clover-affected ewes had been exposed to oestrogenic pasture for 3 seasons of approximately 6 months duration each, while the implanted ewes had been treated for only one 6-month period. The nature of the changes was similar in both groups, indicating that the changes measured have a general utility in estimating the permanent abnormality caused by oestrogen in the ewe. It also indicates that the permanent changes seen in the cervix of ewes with clover infertility are not a peculiar response to phyto-oestrogen, but are part of a general response of the ewe to prolonged exposure to oestrogenic stimulation.

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References

Adams, N R (1976a) — *Res Vet Sci* **21**: 59
Adams, N R (1976b) — *J Comp Path* **86**: 29
Adams, N R (1977a) — *Aust J Agric Res* **28**: 481
Adams, N R (1977b) — *Res Vet Sci* **22**: 216
Adams, N R (1979a) — *Biol Reprod* **21** 1203
Adams, N R (1979b) — *Aust Vet J* **55**: 22
Adams, N R (1986) — *J Reprod Fert* **76**: 727
Adams, N R, McKenzie, P and Sanders, M R (1980) — *Proc Aust Soc Anim Prod* **13**: 491.
Bell, T D, Cassida, L E and Darlow, A E (1941) — *Endocrinology* **28**: 441
Bennetts, H W, Underwood, E J and Shier, F L (1946) — *Aust Vet J* **22**: 2
Davenport, N (1967) — *J Agric WA* **8** (Fourth Series): 83
Ferrington, D G, Fisher, G R, Herron, P and Rowe, M J (1980) — *Neurosci Lett.* **19**: 51.
Heydon, R A and Adams, N R (1977) — *J Comp Path* **87**: 353
Karsch, F J, Dierschke, D J, Weick, R F, Yamaji, T, Hotchkiss, J and Knobil, E (1973) — *Endocrinology* **92**: 799.
Lightfoot, R J (1974) — *Proc Aust Soc Anim Prod* **10**: 113
Lightfoot, R J and Adams, N R (1979) — *J Comp Path* **89**: 367
Schinckel, P G (1948) — *Aust Vet J* **24**: 289
Shier, F L and Rossiter, R C (1949) — *J Dept Agric WA* **26** (Second Series): 111
Wroth, R H and Lightfoot, R J (1976) — *Proc Aust Soc Anim Prod* **11**: 225

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A comparison of blood lead levels in dogs from a lead-mining, lead-smelting, urban and rural island environment

BIOAVAILABILITY

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SUMMARY: A survey of blood lead concentrations was undertaken with 368 dogs from a lead-mining (Broken Hill), lead-smelting (Port Pirie), urban (Adelaide) and rural island (Kangaroo Island) environment. Least squares mean blood lead concentrations differed significantly ($p < 0.001$) between locations and were 1.05, 0.80, 0.38 and 0.32 µmol/L respectively; there were also significant differences between young (0.75) and older (0.52) dogs ($p < 0.01$) and between male (0.71) and female (0.57) dogs ($p < 0.05$). Approximately 14% of dogs from Broken Hill and 10% from Port Pirie had blood lead concentration in excess of 1.7 µmol/L. It is likely that a higher proportion of the dogs at Broken Hill and Port Pirie were asymptomatically suffering from the insidious effects of lead that has not been detected.
Aust Vet J 63: 282-285

Introduction

Little information is available on the environmental impact of lead-related industrial activities such as lead mining and smelting on blood lead levels in dogs. At the Central Veterinary Laboratories, blood of dogs was received for lead assay from various locations throughout South Australia, including the town of Port Pirie (a lead-zinc smelting town) and the New South Wales town of Broken Hill (a lead-silver mining town). Records from the Central Veterinary Laboratories indicate that between 1979 and 1980, 40% of the requests for blood lead assays originated from Broken Hill and of these, 56% were confirmed as cases of lead poisoning. This contrasts with 17% detected in specimens submitted from other areas.

Recently the South Australian Health Commission reported elevated blood lead levels in children from some areas of Port Pirie (South Australian Health Commission 1983), and in 1979 a study undertaken at the Central Veterinary Laboratories showed that sheep grazing in the vicinity of Port Pirie had liver lead concentrations which depended on their relative proximity to the Port Pirie smelters (Koh and Judson 1986). A survey was therefore undertaken to compare blood lead concentrations in dogs from 4 areas with varying involvements in lead production; these were Broken Hill (lead-mining), Port Pirie (lead-smelting), Adelaide (urban) and Kangaroo Island (rural).

Materials and Methods

Sample Collection

Between 1981 and 1983, 9 veterinarians from 4 locations were requested to assist in the collection of blood from apparently healthy dogs presented for routine check-up or vaccination. Usually 2 to 5 mL of blood was obtained by cephalic venipuncture using 21-G needles and 5mL syringes. Samples were subsequently transferred to 5 mL polystyrene blood tubes containing lithium heparin as anticoagulant and were sent to the Central Veterinary Laboratories, Adelaide, for lead assay. Collaborating veterinarians were asked to record the gender, approximate age of the dog and the date on which the sample was collected.

Location

All 4 locations have dry summers and wet winters and a brief description of each is given below:

Broken Hill — A lead-silver mining township with a popu-ion of about 27,000 which covers an area of 68 sq km. ·cated near the border of South Australia/New South Wales d about 350 km north east of Adelaide, the Broken Hill ·neral deposit is one of the largest and richest in the world. nce 1883, about 150 millon tonnes of ore have been mined Broken Hill, with an annual tonnage of about 3 million lew South Wales Department of Mineral Resources 1981). ·e lead is present as galena and marmatite with a lead content 9%. Most of the lead ore is treated at the Port Pirie nelters.

Port Pirie — A lead-smelting township of about 16,000 ·ople with an area of 18 sq km. The town is about 200 km ·rth west of Adelaide. The metal-refining complex at Port ·irie incorporates the world's largest lead smelter. Since 1889, ·e smelters have produced about 12 million tonnes of lead South Australian Department of Mines and Energy 1984) and · 1983, treated about 470,000 tonnes of lead concentrates nd ores (The Broken Hill Associated Smelters Pty Ltd, Work ·roduction List 1983).

Adelaide — The capital city of the state of South Australia ·ith a population of about 980,000 and an area of 1870 sq m. Light industries represent part of the commercial activities n the area.

Kangaroo Island — A rural island of 4410 sq km with a ·opulation of about 4,000 and no mining or manufacturing ·ctivity. The island is about 100 km south west of Adelaide ınd 20 km from the mainland.

Blood Lead Determination

Blood lead was assayed using a modification of the method of Subramanian and Meranger (1981). One hundred µL of blood was dispensed into a 5 mL polystyrene tube containing 400 µL of a mixture of 19 mmol/L di-ammonium hydrogen phosphate and 1.55 mmol/L Triton X-100 (1 : 1 v/v). The contents were mixed and the lead concentration determined with a Varian CRA-90 graphite furnace connected to a Varian AA-775 atomic absorption spectrophotometer. Internal quality control blood materials which had lead concentrations determined by an external laboratory and calibrated against external quality control blood samples were incorporated in each batch of analyses. The accuracy of the lead results was ascertained through monthly participation in an international quality control scheme organised by the Robens Institute, University of Surrey, United Kingdom.

Haematology

Haematocrit values were determined using a micro-haematocrit centrifuge. Haemoglobin content was determined by a standard cyanomethaemoglobin method. Quality control material for haemoglobin* was used to confirm the accuracy of the haemoglobin results. Haematocrit values were not measured on haemolysed samples.

Statistical Analysis

The number of observations within age, gender and location classes varied and were disproportionate. The data were therefore subjected to a least squares analysis assuming a fully fixed model. Initially all interactions were included in the analysis but interaction terms not significant at $p < 0.05$ were deleted from the model and the analysis repeated. McMichael *et al* (1985) suggested that blood lead concentrations followed a log-normal distribution, and the analysis of blood lead concentrations in dogs were repeated with the concentration expressed in log measure. The conclusions were similar and the results presented are for untransformed data. Data with incomplete information on age and gender were excluded from the statistical analysis but were included in Figures 1 and 2.

Results

Samples were collected from 389 dogs from more than 25 breeds; 21 samples were considered unsuitable for the survey which included 12 samples from dogs that showed signs of fitting/vomiting, 3 suspected of having contracted parvovirus, 1 suffering from depression and 5 samples which were at least partially clotted. Of the 12 dogs exhibiting fitting/vomiting symptoms, 9 from Broken Hill had blood lead concentrations (µmol/L) ranging from 0.5 to 13.2 while the remaining 3, from Port Pirie, had blood lead concentrations of 1.5 to 3.0. The location and number of samples included in this report were: Broken Hill, 129; Port Pirie, 89; Adelaide, 77 and Kangaroo Island 73. Sixty-six samples with incomplete information on age and/or gender were excluded from the analysis of variance.

The frequency distribution of blood lead concentration in the 4 canine populations is given in Figure 1. Blood lead concentrations were significantly affected by location ($p < 0.001$), age ($p < 0.01$) and gender ($p < 0.05$) (Table 1). Dogs from Broken Hill and Port Pirie had higher blood lead concentrations than those from Adelaide and Kangaroo Island and those from Broken Hill had higher levels than those from Port Pirie. The highest blood lead values (µmol/L) recorded were 3.4 for Broken Hill, 4.8 for Port Pirie, 1.09 for Adelaide

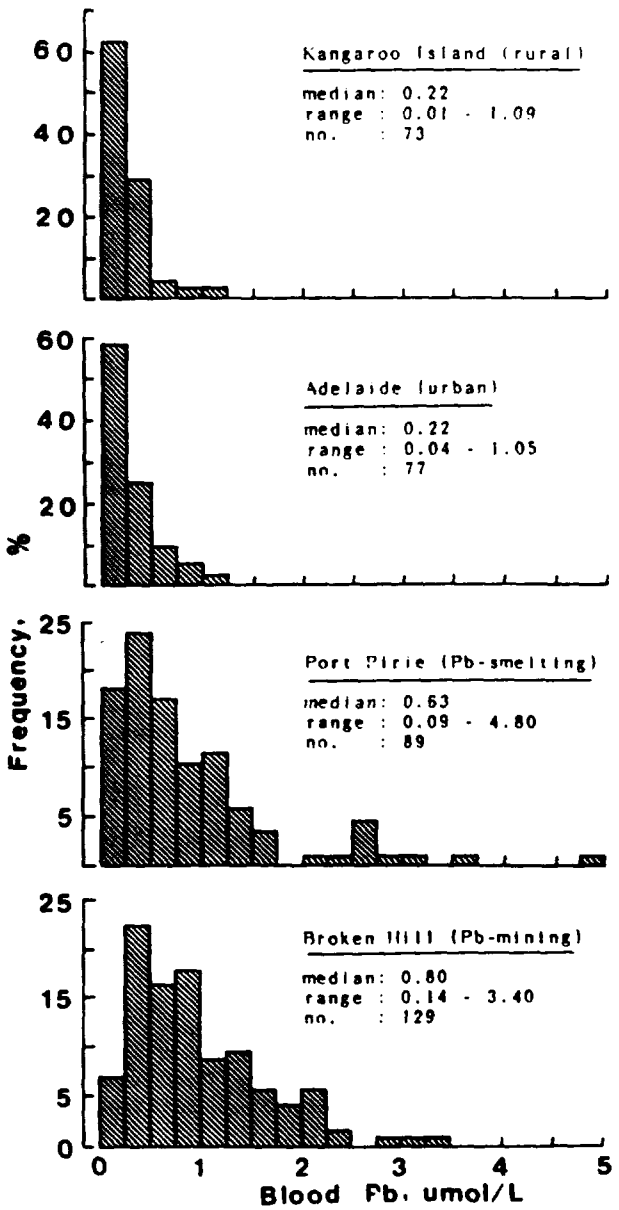

Figure 1. Frequency distribution, median values and ranges of blood lead concentrations in dogs from 4 locations with varying involvement in lead production. Data with incomplete information on age and gender were included.

* 4C®, Coulter Diagnostics, Florida, United States of America

TABLE 1
Effects of location, gender and age on least squares means of blood lead concentration (μmol/L) with the number of dogs in each category given in parenthesis

Location	Blood lead				
	Male	Female	Age (year) ≤ 1	Age (year) > 1	Overall mean
Kangaroo Island	0.39(54)	0.24(17)	0.43(8)	0.20(63)	0.32(71)
Adelaide	0.45(30)	0.31(43)	0.49(23)	0.27(50)	0.38(73)
Port Pirie	0.88(27)	0.73(43)	0.92(32)	0.69(38)	0.80(70)
Broken Hill	1.12(51)	0.98(37)	1.16(20)	0.94(68)	1.05(88)
Overall mean	0.71(162)	0.57(140)	0.75(83)	0.52(219)	0.64(302)

Test of Significance
Location: ***
Gender: *
Age: **

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
None of the interactions is significant ($p > 0.05$).

and 1.05 for Kangaroo Island. Approximately 14% of dogs from Broken Hill and 10% from Port Pirie had blood lead concentration in excess of 1.7 μmol/L. Overall, the least squares mean of 0.71 μmol/L for blood lead levels in male dogs is significantly ($p < 0.05$) higher than the value of 0.57 μmol/L for female dogs. Neuters were excluded from the analysis. Young dogs less than or equal to one year old had a least squares mean blood lead concentration of 0.75 μmol/L which is significantly ($p < 0.01$) higher than that of 0.52 μmol/L for dogs older than one year.

There was no obvious relationship between blood lead concentration and haemoglobin or haematocrit values (Figure 2). However, haemoglobin concentration differed significantly between location ($p < 0.05$) and age ($p < 0.01$) (Table 2). Similarly, haematocrit values were affected by location ($p < 0.001$) and age ($p < 0.01$). There were no first order or second order interactions ($p > 0.05$).

Discussion

Dogs from lead-smelting or lead-mining environments had significantly higher blood lead concentrations even though all appeared clinically normal. More than 10% of the samples from these areas had values which exceeded 1.7 μmol/L for the diagnosis of lead poisoning (Zook 1978). In contrast, about 4% of children in Port Pirie (South Australian Health Commission 1983) were found to have blood lead concentrations which exceeded the 1.5 μmol/L limit recommended by the National Health and Medical Research Council (1979) of Australia as the level of concern. The higher percentage for dogs may be attributable to their flirting activities with non-food objects. This manner of lead contamination is more likely with younger dogs which often possess an irresistible urge to gnaw on almost anything in sight. Our findings that younger

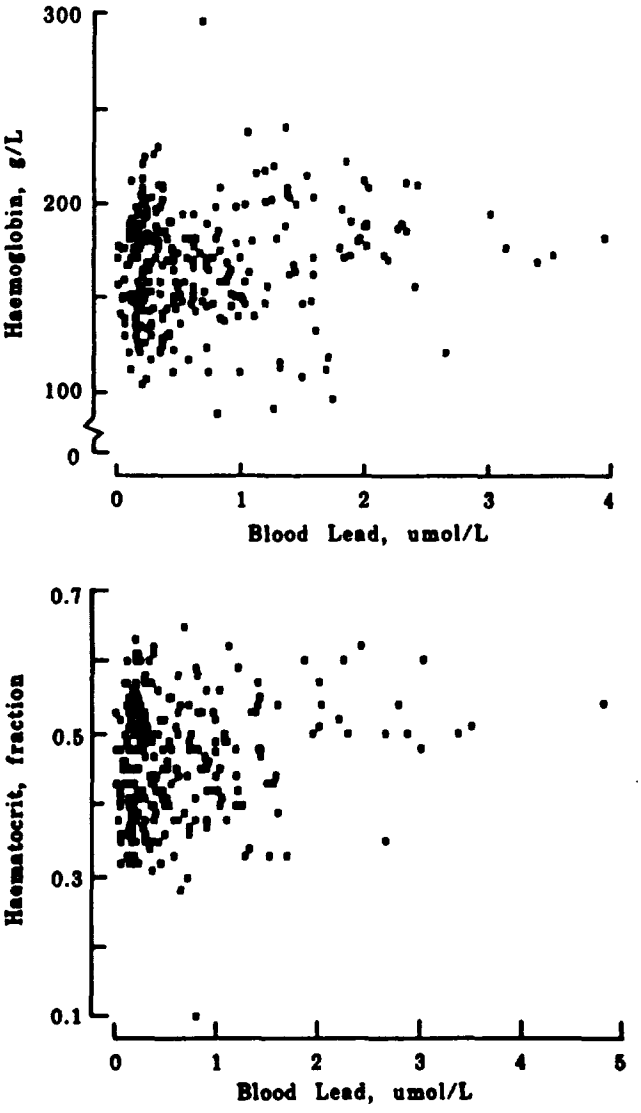

Figure 2. Relationship between blood lead concentration and haemoglobin and haematocrit values. Data with incomplete information on age and gender were included.

TABLE 2
Effects of location, gender and age on least squares means of blood haemoglobin concentrations (g/L) and haematocrit values (fraction) with number of dogs in each category given in parentheses

Location				Age (year)		Gender	
Kangaroo Is.	Adelaide	Port Pirie	Broken Hill	≤ 1	> 1	M	F
Haemoglobin							
173(49)	150(55)	157(49)	165(89)	153(58)	169(164)	161(125)	161(97)
Haematocrit							
0.50(50)	0.42(55)	0.45(70)	0.45(72)	0.44(70)	0.47(177)	0.45(135)	0.45(112)

Test of Significance

	Haemoglobin	Haematocrit
Location:	*	***
Gender:	n.s.	n.s.
Age:	**	**

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.
None of the interactions is significant ($p > 0.05$).

TABLE 3
A comparison of blood lead concentrations (μmol/L) in dogs from this study with those of other reports

Source	No. of dogs	Blood lead
This study:		
		Least squares mean
Kangaoo Island (rural)	71	0.32
Adelade (urban)	73	0.38
Port Pirie (Pb-smelting)	70	0.80
Broken Hill (Pb-mining)	88	1.05
		Mean
Bloom *et al* (1976)	206	0.31 ± 0.27
Thomas *et al* (1975)	89	0.38 ± 0.35
Hayashi and Tsukamoto (1979)	31	0.53 ± 0.53
Reid (1979)	48	0.88 ± 1.48
Zook *et al* (1972)	40	0.92 ± 0.39

logs had higher blood lead values than older ones supports his hypothesis.

Blood lead values from this study and those of others are resented in Table 3. Our findings on dogs from rural (Kangaroo Island) and urban (Adelaide) environments are similar o those of Thomas *et al* (1975) and Bloom *et al* (1976). The esults reported by Zook *et al* (1972) and Reid (1979) appear nusually high for urban environments.

The detrimental effects of lead on haemopoiesis have been locumented (Doss 1978). In humans, there is a negative association between low level of blood lead (0.5 to 1.8 μmol/L) and haemoglobin and haematocrit values (Coutselinis *et al* 979). This was not observed in dogs in the present study or n that of Anderson and Danylchuk (1977) who worked with logs with blood lead concentrations of 0.8 to 3.9 μmol/L ollowing chronic low lead exposure. In our experience, abormal haemoglobin or haematocrit values are rarely observed n dogs exhibiting symptoms of lead poisoning. Although xamination for nucleated red cells or basophilic stippling was ot performed in this survey, we have previously found that ucleated red cells were not observed in 54% of dogs showing linical signs of lead poisoning (Koh 1985).

During and after the survey, we encountered 23 dogs from roken Hill and Port Pirie exhibiting clinical signs of lead oisoning through routine diagnosis. In a previous report (Koh 985) we showed that there is a wide variation in individual usceptibility, the toxic effects of lead and clinical signs of ad poisoning can be observed at a blood lead concentration s low as 0.9 μmol/L. Since more than 10% of the dogs from roken Hill and Port Pirie had blood lead levels in excess of .7 μmol/L, it is likely that a higher proportion of the dogs t these locations is asymptomatically suffering from the indious effects of lead and has not been detected. In children, linical signs of lead poisoning such as fitting and vomiting ave not been reported at a blood lead level of 0.9 μmol/L. This suggests that dogs are more sensitive than children to the toxic effects of lead. Since dogs share a similar living environment with children, a diagnosis of elevated blood lead level and/or lead poisoning in dogs should be regarded as an early warning sign that children may also be at risk. In New Zealand, it is obligatory for veterinarians to report the incidence of lead poisoning in animals to the health authority. We believe that a similar and reciprocal directive from Australian medical and veterinary authorities is desirable and would aid in identifying potential cases of lead poisoning in children and animals. The requirement for a closer liaison in this field is particularly warranted in view of the situation in Port Pirie and Broken Hill.

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References

Anderson, C and Danylchuk, K D (1977) — *Lab Invest* 37: 466

Bloom, H, Noller, B N and Shenman, G (1976) — *Aust Vet J* 52: 312

Coutselinis, A, Trichopoulos, D and Zavitsianos, X (1979) — *Clin Chem* 25: 1339

Doss, M (1978) — *Diagnosis and Therapy of Porphyrias and Lead Intoxication*, International Symposium Clinical Biochemistry, Springer-Verlag, New York

Hayashi, M and Tsukamoto, T (1978) — *Japn J Publ Hlth* 25: 139

Koh, T-S (1985) — *Aust Vet J* 62: 434

Koh, T-S and Judson, G J (1986) — *Bull Environ Contam Toxicol* 37: 87

McMichael, A J, Baghurst, P A, Robertson, E F, Vimpani, G V and Wigg, N R (1985) — *Med J Aust* 143: 499

National Health and Medical Research Council (1979) — *Report 88th Session* Canberra, Australia, p 18

New South Wales Department of Mineral Resources (1981) — *Mining and Exploration at Broken Hill: A Review*, Department of Mineral Resources, New South Wales

Reid, T C (1979) — In *Proceedings of the Second NZ Seminar on Trace Elements and Health*, edited by Aggett, J, Kjellstrom, T, and Crowe, D, The University of Auckland, Auckland, p 90

South Australian Department of Mines and Energy (1984) — *Mineral Resources Review*, No. 154, Adelaide, South Australia

South Australian Health Commission Epidemiology Branch (1983) — *Blood Lead Levels in Port Pirie Children*, Adelaide, South Australia Health Commission Epidemiology Branch

Subramanian, K S and Meranger, J C (1981) — *Clin Chem* 27: 1866

Thomas, C W, Rising, J L and Moore, J K (1975) — *J Am Vet Med Assoc* 167: 996.

Zook, B C (1978) — In *Toxicity of Heavy Metals in the Environment*, edited by Oehme, F W, Marcel Dekker Inc, New York, p 179

Zook, B C, Carpenter, J L and Roberts, R M (1972) — *Am J Vet Res* 33: 891

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